SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

Action number: FA1408
STSM title: Molecular and serological techniques for *Toxoplasma gondii* identification: PCR, ELISA, bioassay, genotyping, magnetic capture, and also molecular techniques used for the diagnosis of muscular cysticercosis: PCR
STSM start and end date: 05/02/2018 to 18/02/2018
Grantee name: Silvia-Diana Borșan

PURPOSE OF THE STSM

The main purpose of this STSM was to get acquainted to the molecular and serological methods used at the National Institute for Public Health and the Environment (RIVM)- Centre for Zoonoses & Environmental Microbiology, Netherlands for the diagnosis of *Toxoplasma gondii*, with a special emphasis on the magnetic capture PCR (MC-PCR) and ELISA protocols.

Another important objective was to learn the molecular methods used for the diagnosis of muscular cysticercosis mainly focusing on *Echinococcus spp.* and *Trichinella spp.,* both being two important zoonosis for the Romanian population and livestock.

Firstly, it was my aim to be able to observe and participate at the MC-PCR protocol because this method it's not yet performed at the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca and it represents an alternative option to the bioassay in quantitative screening of large numbers of meat samples. This method combines homogenised large samples with sequence specific magnetic capture to detect *Toxoplasma gondii* DNA. It has a higher sensitivity than a normal PCR thanks to the large sample volume which is homogenised and has the advantage of reducing the use of experimental animals, thus representing a valuable method to implement in the future research carried out at our University.

Secondly, another purpose of this STSM, as stated above, was to become familiar to the ELISA method used in the serodiagnosis of toxoplasmosis and the protocols used for the detection of *Echinococcus spp.* and *Trichinella spp* – all the steps from the macroscopic examination of a sample, to the PCR design and genotyping of the findings.

Finally, this STSM provided the opportunity for me to acquire a set of practical skills for the laboratory work and also improve my dexterity. I consider gaining experience in this field as a beginner will help my future development as a researcher. Not only this STSM allowed me to better my practical work but also offered me the possibility to really understand the theoretical aspects behind each step of the protocols I was interested in.

In conclusion, I firmly believe that this Short Term Scientific Mission at the National Institute for Public Health and the Environment (RIVM) center for Zoonoses & Environmental Microbiology,

Netherlands will help me perform and implement molecular and serological identification protocols for *Toxoplasma gondii* in rodents, molecular protocols for the diagnosis of muscular cysticercosis at the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca and also increase the quality of my scientific work.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

The training at the National Institute for Public Health and the Environment (RIVM) centre for Zoonoses & Environmental Microbiology, Netherlands was divided into two parts: a theoretical and a practical one, which were included on a everyday basis; additionally after the completion of the scheduled tasks for the day, time was allowed for background research or documentation on the tackled topics.

In the first week (05.02—9,02.2018), the focus was mainly on the serological methods used for the diagnosis of *Toxoplasma gondii*, more precisely the ELISA protocol, this representing the practical part, while the theoretical lectures focused on the molecular techniques used for the detection of *Echinococcus spp* and *Trichinella spp*. Below I will include a detailed work plan for the first week.

Monday (05.02.2018), I started the practical session by identifying the samples that were used in the ELISA assay. The samples we worked on were represented by 46 fox hearts collected in the Netherlands and received by RIVM for further analyses. We individually processed every sample by harvesting 5 g of heart tissue that was cut into small pieces and placed into special containers. The samples were stored at -80°C for 24 hours then moved to 4°C allowing the forming of meat juice, which we later used in the ELISA method. I continued the day by researching and reading literature on the MC-PCR method.

Tuesday (06.02.2018): I attended two lectures of the center for Zoonoses on the „Comparative exposure assesment of ESBL producing E-coli" and „ Cell culture to test effect on meat processing on *T.gondii* viability"; presenting different experiments, results and recommendations and then continued by participating at a lecture on the *Echinoccocus spp.* topic, discussing all the steps that are taken from the macroscopic evaluation to DNA isolation and PCR, DNA sequencing and phylogenetic analysis.

Wednesday (07.02.2018): the practical session was to perform the ELISA assay by using an ELISA ID-VET multispecies kit, discussing and observing all the protocol steps, and evaluation of the results obtained, continued by literature research on this topic.

Thursday (08.02.2018): I participated at two theoretical sessions regarding rodent parasites, *Trichinella* PCR, and the steps involved in the design of a QMRA (quantitative microbial risk assessment) model that stimulates the occurrence of the parasite in wildlife, its transmission dynamics through the food chain from meat inspection to consumption of meat products and consequent human trichinellosis risks.

Friday (09.02.2018): I was involved in three lectures. The first lecture focused on the current state of foodborne parasites on a global level and their impact on the population as showed by the disease burden. The other two lectures included the MC-PCR protocol steps and the QMRA model for meatborne *T. gondii* infection and its implementation in a social-cost benefit analysis for potential toxoplasmosis prevention measures (freezing of meat and increasing biosecurity at pig farms).

The second week (12.02-16.02.2018) was dedicated to performing of the MC-PCR. Being a complex protocol it was divided in multiple stages through the whole week, ending on Friday with the QPCR analysis (explanation of the method and evaluation of the results).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

ELISA IDVET multispecies

The protocol was carried out following the manufacturer's specifications. The optical density was read and recorded at 450 nm, and the results were evaluated as stated in the kit. From the 46 fox samples that we analysed, 43 were confirmed positive for *Toxoplasma gondii* antibodies , and 3 negative.

MC-PCR protocol

For the MC-PCR we took into study a number of 24 samples.The first 12 samples were represented by negative bovine heart tissue (50g), from the Netherlands, tested in the 2015 EFSA study. These samples were divided into 3 groups of 4 samples each. Every group had 3 samples spiked with a known number of tachyzoites: 250, 2500 and 25000 tachyzoites/sample and a negative sample (blank). The next 8 samples were represented by randomly selected positive fox samples for the ELISA assay. Finally, the last 4 samples were represented by the positive controls: 250, 2500 and 25000 tachyzoites concentration and the negative control.

After the sequence specific magnetic capture of DNA all the samples were analysed by a real-time PCR targeting the 529-bp repeat element. A competitive internal amplification control (CIAC) was added to enable identification of false negative PCR results. For each cycle the fluorescence-by-cycle curve was used to calculate the crossing point (Cp) at which the second derivative was at its maximum. All samples with a Cp-value that showed a smooth exponential amplification curve were considered positive, all samples without a Cp-value but a positive CIAC-PCR were scored negative. From our first 12 bovine samples, all the spiked samples were scored positive with similar Cp-values for identical spikes, and the blanks were negative. From the 8 (ELISA positive) fox samples analysed, 7 of them were positive and 1 negative, all 3 positive controls tested positive and the blank one negative.

Our results show a high seroprevalence of *T. gondii* infection in the tested foxes, paired with similar results by using a molecular assay. This leaves room for further investigations regarding the seroprevalence of *T.gondii* infection in foxes in the Netherlands, and their role in the life cycle of this parasite.

FUTURE COLLABORATIONS (if applicable)

In Romania information about the seroprevalence of *T.gondii* in livestock or wildlife is rather vague or scarce, also there is limited research regarding the molecular epidemiology and genetic diversity of *T.gondii* genotypes, therefore leaving space for further investigations in this field. I received some advice for my my PhD project in Romania not only to focus on *T. gondii* in rodents but also in livestock species and to study the transmission from wildlife to livestock.

Taking into consideration the above mentioned advantages of the MC-PCR method and in the possible event of future implementation of this assay at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, we will establish a collaboration for further research on this topic between our University and the researchers team involved in this STSM from the National Institute for Public Health and the Environment (RIVM) center for Zoonoses & Environmental Microbiology, Netherlands.